

# DNA Mechanics Affected by Small DNA Interacting Ligands

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## Abstract

We have investigated the mechanics of individual DNA strands exposed to DNA binding ligands. The interaction of these agents with individual dsDNA strands measured by optical tweezers clearly indicates the ligand-DNA binding mode. As expected, if the compound is intercalating then an increase of contour length is detected. Groove binders affect the overstretching capabilities of the formerly "naked" dsDNA

strand. We interacted SYBR® Green I with naked dsDNA. The binding mode of this compound, which is used for nucleic acid gel staining, is not known. The mechanics of the interaction of SYBR® is revealed by optical tweezers experiments. The force extension curves on single dsDNA fragments show a groove-binding mode, which does not affect the contour length of the molecule but significantly alters the overstretching behaviour of the dsDNA.

## Introduction

Small nucleic acid binding agents are used as DNA staining reagents in molecular biological techniques [1]. These agents bind to the DNA in different manners: intercalation between the base pairs, within the minor or major grooves, and by non-classical modes [2]. For typical DNA staining experiments (e.g. gel electrophoresis), the binding properties of these compounds affect the fluorescence signal to noise ratio significantly. For instance, it is known that Ethidium Bromide (MW 394.32; CAS 1239-45-8), which has to intercalate between the individual stacked base pairs, cannot be used if the amount of DNA is typically less than tens of nanograms. In contrast, other compounds such as SYBR® Green I (Molecular Probes, Eugene, OR), which bind to dsDNA with great specificity, are able to reveal DNA amounts of two orders of magnitudes lower weight.

In addition, small DNA binding ligands have great importance in treatment of genetic, oncogenic and viral diseases [3]. They can for instance act as probes for nucleic acid damage and structure [4]. The activity of a variety of naturally occurring and man-made antibiotics has been linked to their ability to bind the minor groove of DNA. Sequence-specific DNA-binding small molecules that can permeate human cells potentially could regulate transcription

of specific genes. For instance Dickinson et al. [5] showed that these synthetic ligands specifically inhibit DNA-binding of transcription factors, and therefore provide a general approach for regulation of gene expression, as well as a mechanism for the inhibition of viral replication.

There are different approaches to get insight into the binding interaction, affinity and specific amount of molecules bound per base pair among which are x-ray diffraction and NMR studies [6,7]. In a recent study, Coury et al. [8] presented a procedure to detect these properties by measuring the contour length of the dsDNA molecule by scanning force microscopy. They incubated the bare DNA molecules with the specific agent and subsequently deposited the modified molecules onto a mica surface and investigated the amount of extension relative to the contour length. For their studies it was shown that the fraction of bound molecules could be estimated and an affinity could be determined by subjecting the DNA to various amounts of ligands. Such an approach reveals some of these parameters, but has the drawback that molecules have to be placed onto a surface in order to be accessible to the SFM imaging. It was shown [9,10] that the deposition of DNA onto mica is allowing the dsDNA molecule to adhere to the surface in an equilibrated manner, but in the subsequent study [10] it was experimentally explored that the dsDNA is partially changing its conformation from B-form DNA to A-form. A significant influence of the surface vicinity onto the binding behavior of the small reagents to DNA has to be included, and therefore, such measurements using the SFM can only reveal general trends upon reagent binding. If agents are investigated which bind to the grooves of the DNA, then an increase of contour length is not occurring. The specific binding of the groove-binding compound has to be examined by competition experiments with intercalating agents to prove an interaction.

**Fig. 1. A)** Scheme of the double beam optical tweezers showing the counter propagating laser beams ( $\lambda = 830$  nm) and the beads held by the micropipette and the OT within the fluid chamber. **B)** A typical picture obtained during the optical tweezers experiment. A bead (3.1 microns in diameter) coated with streptavidin receptors is held by suction on a micropipette. A second bead (in the center of the image, 2.9 microns in diameter) is trapped in three dimensions using two counter propagating laser beams. Single dsDNA molecules are covalently attached to this latter bead and have a free biotinylated 3' end (schematically shown (not to scale)). For a typical experiment, the micropipette is moved with nanometer accuracy close to the streptavidin bead till some deflection of the laser beam on a position detector is observed. In this case, the dsDNA single molecule is linked to the streptavidin receptor through a streptavidin-biotin bridge.

Also, force spectroscopy was performed with SFM to discriminate small molecule DNA binding mode [11]. It was shown that the mechanical properties are greatly affected



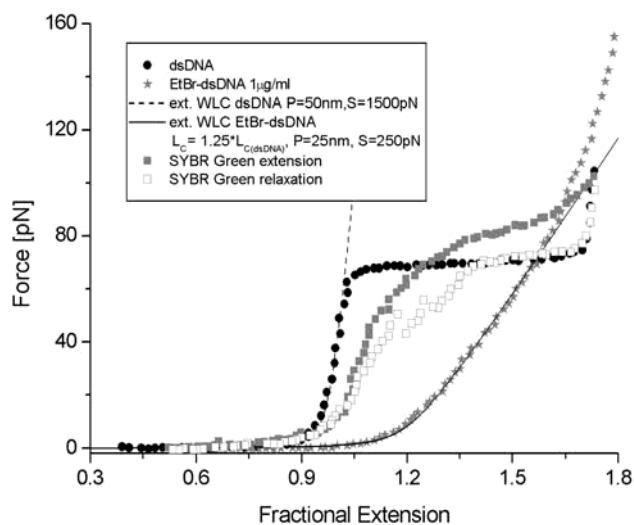
when small molecules interact with DNA. The force versus extension curves show typical and different behaviours for cross-linking (cisplatin), minor groove binder (bernil), and intercalating [ethidium bromide (EtBr)] molecules. Thus, it was emphasized that force spectroscopy could be used as a fast and reliable tool for screening purposes.

To further investigate the binding mode of such small interacting molecules, we present in this paper OT experiments. While SFM is best suited when overall mechanical properties have to be investigated, OT is a technique of choice to reveal small changes in mechanics (persistence length, stretch modulus). In this study, we show experiments performed on both EtBr and SYBR® green (i.e. a compound of which the binding mode is not clearly indicated). In agreement with SFM [11], we observe characteristic changes for each DNA binding molecule. Both the persistence length and the stretch modulus of DNA show a significant decrease upon interaction with such agents. Moreover, by applying a constant force feedback while injection of the agents, we were able to determine binding kinetics.

## Experimental Section

### Modification of dsDNA and Coupling to Beads

For typical optical tweezers experiments, a micron-sized bead (BangsLabs, Fishers IN) is generally coated with some receptor (e.g. antibodies or streptavidin) and placed onto a micropipette that can be moved using a piezoelectric element. Then a second set of microspheres is injected in the fluid chamber and one of them is trapped. These spheres have single dsDNA molecules attached with one end to the chemically activated surface, exposing on the other dsDNA end a ligand (e.g. biotin) into the solution. The experiment then just consists of approaching the pipette close enough to the bead in the trap till some force is felt onto a detector (Fig. 1 A and B). Due to the high affinity of biotin-streptavidin ( $K_D > 10^{-14} \text{ M}^{-1}$ ) the molecular recognition of this ligand-receptor interaction is easy to achieve. In contrast, an efficient site directed coupling of single molecules to beads is generally more demanding. Although there are different approaches to attach DNA molecules to chemically modified beads, the best is certainly to use a site directed covalent coupling for one end only because it allows making stock solutions of material and to reach high forces while applying external tension. For instance, an antigen-antibody bridge will rupture at forces above  $\sim 50 \text{ pN}$  (in typical slow pulling tweezers experiments). Site directed covalent coupling of dsDNA to amino beads was performed using a procedure similar to the one described in [12]. In brief, we use a commercial dsDNA circular plasmid ( $\rho\text{TYB1}$ , 7477 base pairs (NEB, Beverly, MA)). The plasmid DNA was then digested with a restriction enzyme (BSA1), which cuts only once in a non symmetrically manner. In other

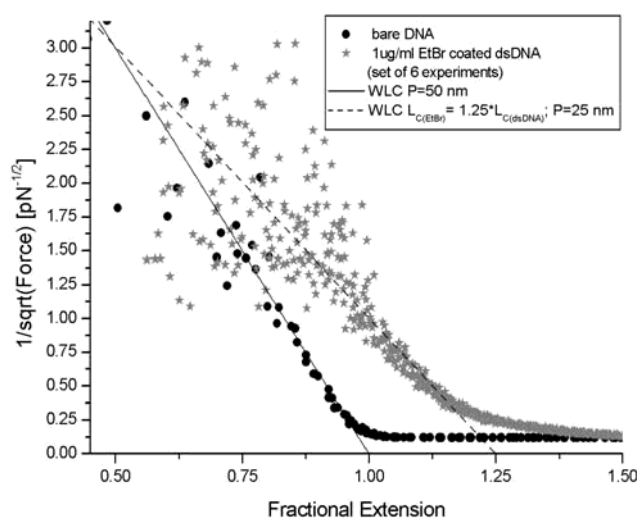


**Fig. 2.** Typical force versus fractional extension  $x/L$  curves obtained for bare dsDNA (circles); and EtBr (stars). SYBR® Green I dsDNA (squares, full square extension, hollow squares relaxation), Also shown is the fit to the data using an extensible worm like chain model (Eq. 1) (dashed lines). From this fit; relevant enthalpic parameters (stretch modulus) are obtained.

words, two different non-palindromic overhangs are obtained after a single enzymatic digestion that can be subsequently modified in two different ways. At one end of the digested plasmid DNA, biotinylated nucleotides (Invitrogen, Basel CH) were incorporated using the Klenow exo--polymerase enzyme (NEB, Beverly, MA). On the other end small thiol modified dsDNA extensions (Microsynth, Balgach CH) were ligated. After intensive cleaning, we end up with modified dsDNA molecules with biotin groups at their 3' end and a thiol group at the 5' end. Covalent coupling of as-modified dsDNA molecules to amino-beads (BangsLabs, Fishers IN) was achieved through a standard SMCC cross linker (Pierce, Rockford, IL) as described in [12].

### Optical Tweezers

The implementation and description of the optical tweezers instrument used to perform the experiments have been presented in details in [13]. Basically, it consists of a dual beam apparatus, i.e. two counter propagating laser beams which share coincident foci. In contrast to single beam optical tweezers, - our instrument directly measures the change in light momentum flux when a trapped object experiences a force. This instrument, therefore, has to be calibrated once since local parameters do not affect the force readings. In



**Fig. 3.** The inverse of the square root of the force  $F$  as a function of the fractional extension  $x/L$  (squares, bare dsDNA; circles, dsDNA after EtBr intercalation). The linear dependence observed for forces below  $\sim 10$  pN is typical of a non-extensible worm like chain (WLC) behavior. The persistence length (contour length) is deduced from an extrapolation to zero extension (force). Because the point where the DNA is attached onto the streptavidin bead is unknown; the contour length has to be adjusted according to the length of the linearized dsDNA plasmid. The jittering observed above  $\sim 1.5$  indicates where thermal noise fluctuations dominate the signal.

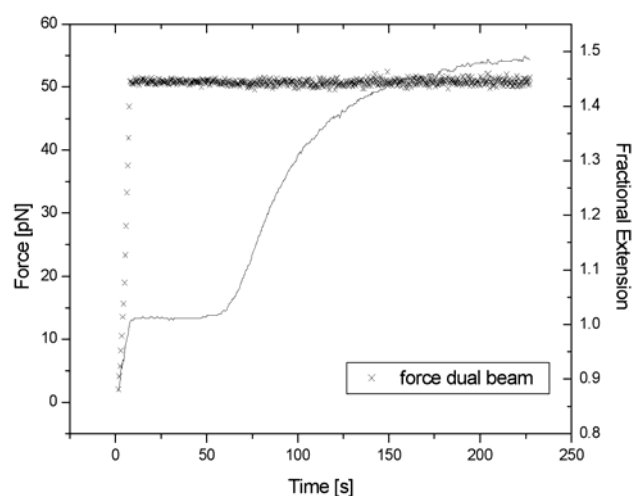
addition the dual beam optical tweezers has a high trapping efficiency, which is typically three times larger than in a conventional single beam optical tweezers setup.

## Results

Although AFM based techniques have been applied in the past to investigate mechanical properties of single polymers [14,15], intrinsic relevant parameters such as the persistence length are not accessible to this technique, mainly because of the large thermal noise of commercial AFM cantilever. Optical tweezers have, however, typical force resolution of about 0.3 pN and overcome limitations of standard AFM devices. This is of prime importance in our study since only slight changes are expected while interacting small DNA binding molecules.

### Mechanics of dsDNA

To check the integrity of our bare dsDNA, we show in Fig. 2 a typical force  $F$  versus extension  $x$  curve (150 mM NaCl, Hepes buffer pH 7.5). As expected, the dsDNA (circles) shows an



**Fig. 4.** Incorporation of EtBr into a dsDNA single molecule followed in real time. For this experiment, a bare dsDNA was first pulled beyond its entropically regime up to 50 pN (0 to 5 seconds) along the X-axis (as defined in our experiment). Then, a flow of EtBr (2.5  $\mu$ M) was applied in the chamber (15 pN along the Y direction, i.e. perpendicular to the pulling direction) and the force along the X-axis was kept constant at 50 pN. When EtBr arrives in the vicinity of the single dsDNA molecule (after 50 seconds), the bead end to end distance starts to increase, indicating intercalation of EtBr into adjacent DNA base pairs. After 150 seconds, the bead end-to-end distance does not show any significant dependence, which reveals maximum incorporation of EtBr. Crosshairs, force (pN); line, fractional extension.

overstretching plateau at 68 pN (S-transition), in excellent agreement with previous studies [16]. Although the origin of the transition is subject of some debate [16,17], the force versus extension curve for forces below 60 pN can be well described by an extensible worm like chain model (WLC) [18], using:

$$x/L = 1 - 0.5(k_B T / FA)^{1/2} + F/S \quad (1)$$

where  $A$  is the persistence length,  $S$  the stretch modulus of the molecule,  $L$  the contour length, and  $k_B T = 4.1$  pN·nm at room temperature. At small forces (typically smaller than 5 pN), only entropy should contribute to the observed mechanics of DNA and we can - to a good approximation - neglect any enthalpic elastic terms ( $S$ ) in Eq. 1. In this case, a linear fit of  $F^{-1/2}$  (Fig. 3) as a function of the extension gives a robust estimate of the persistence length  $A$ . We find an  $A$  value of 50 nm, consistent with previous studies [16,18]. Knowing this latter parameter and the contour length of the molecule, the stretch modulus of the dsDNA can be easily

obtained from a fit of the force versus fractional extension using Eq. 1. We obtain an  $S$  value of 1500 pN using our standard chamber buffer.

## Mechanics of dsDNA Interacting with Small Molecules

In the previous paragraph we measured the individual properties of "naked" dsDNA in order to have a molecular "ruler", to which we can compare the subsequent experiments. Next, we investigated the interaction of the bare dsDNA with ethidium bromide (EtBr). For such agents that directly intercalate between adjacent base pairs we expect to observe great changes in the mechanical properties. Therefore, by measuring the end-to-end distance of the molecule, while maintaining a constant force, we can directly follow the uptake kinetics of DNA binding agents. This way we ensure that the DNA molecule is covered to its maximum binding capabilities at a specific concentration of reagent. We present in figure 4 the results of such a force feedback experiment at  $\sim 50$  pN. The flow force of EtBr for this experiment was 15 pN in the orthogonal direction [2.5 mM], not affecting the force feedback on the dsDNA molecule. Incorporation of EtBr is completed after  $\sim 150$  s for 2.5  $\mu$ M at the chosen flow rate.

In figure 2 the mechanical properties of such an EtBr intercalated dsDNA molecule is shown (stars). A fitting procedure identical to the one describe above reveals values of 25 nm and 250 pN for the persistence and stretch modulus respectively. In addition, we observe a change of contour length of about 25 % due to the intercalation of EtBr. According to [19], the increase of length per EtBr molecule is 0.34 nm. Observing the increase of length directly from the measurement, we can conclude that, on average, every fourth base pair has intercalated EtBr, which can be difficult to access using scanning force spectroscopy measurement. Furthermore, from the parameters extracted, we see that the intercalation of dyes is greatly affecting the mechanics of bare dsDNA. Namely the decrease of persistence length by factors two and the reduction of the stretch modulus of six. We should mention that an applied tension on the double strand DNA during the intercalation did not bias the amount of uptake of binding agents. For instance, feedback on the force at 50 pN or at 0 pN does not change any mechanical properties.

Additionally, in figure 2 the force versus extension of a dsDNA coated with SYBR<sup>®</sup> Green I is shown (squares). As visible in the graph, the interaction of the SYBR<sup>®</sup> Green I with dsDNA doesn't alter the contour length of the molecule, but slightly decreases the persistence length (40 nm) and the stretch modulus (500 pN) of the DNA molecule during the extension cycle. During the relaxation of the molecules in presence of SYBR<sup>®</sup>, we repeatedly observe hysteresis of the force versus extension experiment. The overstretching

plateau of the interacting dsDNA is affected during extension and during relaxation. Extension of the molecule shows a short overstretch plateau occurring at higher force values  $\sim 80$  pN but almost no cooperativity as in the S-transition of bare dsDNA is observed. The force extension curve is then "merging" with the one from bare dsDNA after the S-transition. During relaxation of the SYBR<sup>®</sup>-dsDNA complex, again an overstretch plateau is observed which indicates either that some SYBR<sup>®</sup> molecules unbound during the high force applied to the dsDNA molecule or that intermolecular forces in-between SYBR<sup>®</sup> molecules have been ruptured, which frees parts of the bare backbone of the underlying dsDNA molecule. During the next pulling cycle, the force versus extension curve follows on a comparable path and shows the same hysteresis.

## Conclusion

Using optical tweezers experiments, we were able to measure directly the kinetics of binding of small ligands to dsDNA. By recording a force versus distance experiment, we are able to extract the mechanical parameters of the modified dsDNA molecule directly. The parameters obtained indicate the way of binding, if intercalation occurs, then the contour length is affected. Additionally, we can determine the occupancy of the ligand on the DNA from such measurements and see how the native mechanics of the molecule is altered. If compounds bind to dsDNA which aren't intercalating, then the binding is directly revealed in the way the modified dsDNA is going through its overstretch transition. Such experiments can give direct insight into the binding of small ligands to DNA and can be of great importance for a general only screening of other compounds.

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## References

- [1] Haugland R.P., Handbook of fluorescent probes and research chemicals. Molecular Probes, Inc., Eugene OR, 1996.
- [2] Lipscomb L. A., Zhou F. X., Presnell S. R., Woo R. J., Pee, M. E., Plaskon R. R. Williams L. D. *Biochemistry* **35** (1996) 2818-2823.
- [3] Haskell C.M., Cancer Treatment, Saunders, Philadelphia 1990.
- [4] Williams, L. D., Goldberg, I. H. *Biochemistry* **27** (1988) 3004-3011.

- [5] Dickinson L. A., Gulizia R. J., Trauger J. W., Baird E. E., Mosier D. E., Gottesfeld J. M. Dervan P. B. Proc. Natl. Acad. Sci. USA, **95** (1998) 12890- 12895.
- [6] Coste F., Malinge J. M., Serre L., Shepard W., Roth M., Leng M., Zelwer C. Nucleic Acids Res. **27** (1999) 1837-1846.
- [7] Gelasco, A., Lippard, S. J., Biochemistry **37** (1998) 9230-9239.
- [8] Coury J.E., McFail-Isom L., Williams L.D., Bottomley L.A. Proc. Natl.Acad.Sci. USA **93** (1996) 12283-12286.
- [9] Rivetti C., Guthold M., Bustamante C., J. Mol. Biol. **264** (1996) 919-932.
- [10] Rivetti C., Codeluppi S., Ultramicroscopy **87** (2001) 55-66.
- [11] Krautbauer R., Pope L.H., Schrader T.E., Allen, S. H.E. Gaub Febs Lett. **510** (2002) 154-158.
- [12] Hegner M., Single Mol. **1** (2000) 139-144.
- [13] Grange W., Husale S., Güntherodt H.-J., Hegner M., Rev. Sci. Instr. **73** (2002) 2308-2316
- [14] Rief M., Clausen-Schaumann H., Gaub H.E., Nature Struct. Biol. **6** (1999) 346-349.
- [15] Fisher T. E., Marszalek P.E., Oberhauser A.F., Carrion-Vazquez M., Fernandez J.M. J. Physiol.-London **520** (1999) 5-14.
- [16] Smith S. B., Cui Y., Bustamante C., Science **271** (1996) 795-798.
- [17] Rouzina I., Bloomfield V.A. Biophys J. **80** (2001) 882-893
- [18] Hegner M., Smith S.B., Bustamante C., Proc. Natl. Acad. Sci. USA **96** (1999) 10109-10114.
- [19] Lerman L.S. J. Cell. Comp. Physiol. **64** (1964) 1-18.